

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing as part of a fusion protein in a bacterium a DNA molecule encoding a mini-proinsulin compound of the formula:



(b) liberating said mini-proinsulin compound from said fusion protein;

(c) folding and forming disulfide bridges in said mini-proinsulin compound; [and]

(d) incubating said mini-proinsulin compound with trypsin at a pH of about 6.8 to produce mono-Arg-insulin, under conditions where no crystals are formed; followed by

(e) precipitating the mono-Arg-insulin.

22. (Three times amended) A method for the preparation of insulin which comprises:

(a) expressing as part of a fusion protein in a bacterium a DNA molecule encoding a mini-proinsulin compound of the formula:



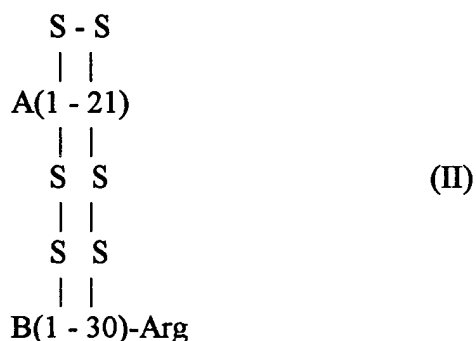
in which B(1-30) and A(1-21) denote the B and A chains of insulin;

- (b) liberating said mini-proinsulin compound from said fusion protein;
- (c) folding and forming disulfide bridges in said mini-proinsulin compound; [and]
- (d) simultaneously incubating said mini-proinsulin compound with trypsin and

carboxypeptidase B at a pH of about 6.8 to produce insulin, under conditions where no crystals are formed; followed by

- (e) precipitating the insulin.

25. (Three times amended) A method for the preparation of a mono-Arg-insulin compound of formula II



in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

- (a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises



DORSCHUG et al. -- Serial No. 08/402,394

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg -,

to a peptide which stabilizes the fusion protein;

(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide;

(c) folding and forming disulfide bridges in said mini-proinsulin compound; [and]

(d) incubating said mini-proinsulin compound with trypsin at a pH of about 6.8 to produce mono-Arg-insulin, under conditions where no crystals are formed; followed by

(e) precipitating the mono-Arg-insulin.

26. (Three times amended) A method for the preparation of insulin which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg -,

to a peptide which stabilizes the fusion protein;

(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide;

(c) folding and forming disulfide bridges in said mini-proinsulin compound; [and]

LAW OFFICES

FINNEGAN, HENDERSON,  
FARABOW, GARRETT  
& DUNNER, L.L.P.  
1300 I STREET, N.W.  
WASHINGTON, DC 20005  
202-408-4000